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EFFICIENCY OF SOME PLANT EXTRACTS, NATURAL OILS, BIOFUNGICIDES AND FUNGICIDES AGAINST ROOT ROT DISEASE OF DATE PALM

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ABSTRACT

Several soilborne fungi were isolated from root rots of date palm trees and offshoots, including: *Fusarium oxysporum*, *F. moniliforme*, *F. solani*, *Thielaviopsis paradoxa*, *Botryodiplodia theobromae* and *Rhizoctonia solani*. *In vitro* testing, the efficacy of plant extract Marjoram at 100% was the most effective against pathogenic fungi, while garlic as essential oil at 500 ppm and jojoba as fixed oil at 500 ppm were the most effective against pathogenic fungi. Plant-Guard at 3.5 ml/L was the most effective against pathogenic fungi. Topsin M70 was the most effective against pathogenic fungi *in vitro*. In greenhouse Topsin M70 was the most effective to decreased root rot of date palm.

Key Words: Date palm, soil borne fungi, root rot, plant extracts, essential oils, fixed oils, biofungicides, fungicides.

INTRODUCTION

The most important pathogens of root rot (decline) disease in date palm trees and offshoots (*Phoenix dactylifera* L.) were *Fusarium oxysporum*; *F. solani*; *F. moniliforme*; *F. equiseti*; *Phoma* sp.; *Alternaria* sp.; *Cladosporium* sp.; *Macrophomina phaseolina*; *Thielaviopsis paradoxa*; *Diplodia phoenicum*; *Phomopsis phoenicola*, *F. semitectum*, *Rhizoctonia solani* and *Chaetomium* sp. (El- Deeb et

al., 2007; Samir *et al.*, 2009; Baraka *et al.*, 2011). Synthetic fungicides are helpful to sustain crop production by protecting plants from fungal diseases, but resistance to fungicides is one of critical causes of poor disease control of agriculture. Therefore developing alternative agents for the control of pathogenic fungal diseases in plants (Aguin *et al.*, 2006). Is needed several researchers have shown that plant extracts may control anamorphic fungal plant pathogens (Parveen and Kumar, 2000; Bhatm, 2001; Agrios, 2005). Biological control of fungal plant pathogens appears as an attractive and realistic approach, and numerous microorganisms have been identified as biocontrol agents. A considerable role in limiting the populations of these pathogenic fungi inhabiting the aboveground parts of plants is played by antagonistic microorganisms. Such properties are first of all exposed by the fungi *Trichoderma* and *Gliocladium*. (Kaewchai and Soytong, 2010) mentioned that application of different bio-agents to the soil under greenhouse conditions, to control the root rotting fungi revealed that the plant irrigated with water, containing *B. subtilis* (2.5 g/l⁻¹) decreased the disease severity over control. *Trichoderma* is recognized as a successful saprophytic fungus, besides being reported as a parasite on other fungi. Dhahira and Qadri (2010) found that the conventional chemical control measures are unable to provide total control. Hence, antagonistic microorganisms were evaluated individually and in combinations for their bio-control potential against *Fusarium* spp., which caused a serious problem due to rampant incidence of root rot disease in mulberry trees (*Morus indica* L.). The best treatment was a combination of *Trichoderma* spp. and the treatment was more effective, when the application of biocontrol agents were taken up at the initial stages of infection. Ammar (2007) reported that, applied Carbendazim fungicide as soil drench was the most effective in disease severity when used against *Fusarium oxysporum*, which caused corm rots and wilt of banana. Srivastava *et al.* (2010) reported that, the radial growth of *F. oxysporum* f.sp. *psidii* was inhibited at high and low concentrations of carbendazium 50% WP. The aim of this research was to examine some plant extracts, natural oils, biofungicides and fungicides against root rot disease of date palm.

MATERIALS AND METHODS

Source of pathogenic fungi

A pathogenic fungi isolates of *Fusarium oxysporum* Schlecht, *Fusarium solani* (Mort.) sacc, *Fusarium moniliforme* Sheldon, *Botryodiplodia theobromae* Pat, *Thielaviopsis thielavioides* Peyr. and *Rhizoctonia solani* Kuhn. obtained by Baraka *et al.* (2011) from infected root rot of date palm (*Phoenix dactylifera* L.) and confirmed as pathogenic fungi were used in present study.

Plant extracts

Four plant materials were used in this experiment namely, Basil (*Ocimum basilicum* L.), Marjoram (*Origanum majorana* L.), Peppermint (*Mentha piperita* L.) and Spearmint (*Mentha spicata* L.) as dried leaves. Plant materials were obtained from Gelcy Agro Organic Co. Giza, Egypt. Derided leaves (2-8mm) were further homogenized into a paste of each leaf with a blender and extracted by two methods (cold or hot extract) according to (Wokocha and Okereke, 2005).

Antifungal activity of plant extracts on the mycelial growth inhibition of the pathogenic fungi *in vitro*

Five concentrations of each test plant extracts, *i.e.* (0, 25, 50, 75, and 100%) were used. Effect of plant extracts (cold or hot) on mycelia extension of the pathogenic fungi were obtained by placing one disc (5 mm diameter) of active cultures in each of five Petri dishes with PDA medium and leaf extract. The control was set up with sterile distilled water. Five replicates plates of leaf extract agar per isolate were incubated at (25±2°C). Mycelial growth inhibition is taken as growth of the fungus on the leaf extract agar expressed as percentage of growth on the PDA as followed: %MGI = DC – DT / DC X 100 Where: %MGI = % Inhibition of mycelial growth, DC = diameter of control, DT = diameter of test. Extracts were rated for their inhibitory effects using the scale described by (Sangoyoni, 2004).

Natural oils

Pure-grade of essential oils, *i.e.* onion (*Allium cepa* L.), garlic (*Allium sativum* L.) and clove (*Syzygium aromaticum* L.) were obtained from Haraz Factory for Natural Oils, Cairo- Egypt. Pure-grade of fixed oils, *i.e.* Rocket (*Eruca sativa* L.), Sesame (*Sesamum indicum* L.) and Jojoba (*Simmondsia chinensis* L.) were obtained from El Baraka Factory for Natural oils, Hurghada- Egypt.

Effect of natural oils on mycelial growth inhibition *in vitro*

Antifungal activity on pathogenic fungi colony development were obtained by dilution method (0, 25, 50, 100 and 500 ppm) of essential or fixed oils in the appropriate culture media-PDA. The oils were dissolved in 5% Tween 20 and added to the 20 ml of PDA before solidified into Petri dish. One disc (0.5 cm diameter) of mycelial plug, taken from the edge of active cultures, was placed into the Petri dish. Controls consisted of 5% Tween 20 mixed with PDA and were handled similarly with the exception of the volatile treatment. Five replications of each treatment were tested and the average was calculated. Control sets were simultaneously run without using the essential or fixed oils. Inhibition and efficacy were measure as described before.

Bioassay of commercial bioagents formulation on growth inhibition of pathogenic fungi *in vitro*

Four commercial bioagents were used in this experiments viz., Bio-zeid (*Trichoderma album*) 10×10^6 spores/ml, Bio-Arc (*Bacillus megaterium*) 25×10^6 cell/ml, Plant Guard (*Trichoderma herzianum*) 30×10^6 spores/ml, Rhizo-N (*Bacillus subtilis*) 30×10^6 cell/ml. Three concentrations of each test commercial bioagents, *i.e.*, 1.25, 2.5 and 3.5 g / L. for Bio-zeid and Bio-arc; 3, 4 and 5 g / L. for Rhizo-N while; 1.25, 2.5 and 3.5 ml / L. for Plant guard, were used. Bioassay of bioagents activities on pathogenic fungi colony development were obtained by dilution method different concentrations of bioagents in the appropriate culture media-PDA. The bioagents were dissolved in sterile distilled water (SDW) and added to the 20 ml of PDA before being solidified into Petri dish. One disc (0.5 cm diameter) of mycelial plug, taken from the edge of active cultures, was placed into the Petri dish. Controls consisted of SDW mixed with PDA and were handled similarly with the exception of the fixed oil treatment. Five replications of each treatment were tested and the average was calculated. Control sets were simultaneously run without using the bioagents formulations. Inhibition and efficacy were measure as described before.

Chemical control

Six fungicides were used in this study viz., Thiophanate methyl (Topsin M70/WP 70%), Carbendazime (Kema-Z/WP50%), Carboxin thiram (Vitavax/WP75%), Hymexazol (Tachigaren/WP30%), Flutolanil (Moncut/WP25%) and Pencycuron (Monceren WP25%).

Effect of fungicides on mycelial linear growth of pathogenic fungi

Six concentrations of each test fungicide, *i. e.* 5, 10, 50, 100, 500 and 1000 ppm based on the active ingredient of each fungicide were used *in vitro*. Effect of fungicides on mycelia extension of the pathogenic fungi was obtained as mentioned before.

Date palm root rot disease control with different treatments in greenhouse

Biological control (abiotic or biotic) and fungicides were used in these experiments. The most effective bioagent (abiotic or biotic) and chemical formulation were applied in greenhouse according to the results *in vitro*. The most effective treatments *in vitro* of plant extracts, natural oils, biofungicides and fungicides were used in two experiments. All treatments were treated as a soil drench to control root rot diseases of date palm seedlings var. Zaghloul. The experiment was divided into two treatments according to the treating time of the control agent and the pathogenic fungi. In section 1, of treatment, the control agent was applied three days before pathogenic fungal inoculation. In section 2, of treatment the control agent was applied three days after the pathogenic fungal inoculation. Control treatments pathogenic fungal inoculation without any treatments. Data were recorded after complete death of the treated plants in the control treatment. The recorded data were calculated as disease severity while was previously explained at the end of the pathogenicity test according to Baraka *et al.*, (2011). The calculated data were converting into reduction in disease severity percentage according to Abdalla *et al.* (2000) and Abdullah *et al.* (2003a). The experiments were repeated twice. The data was displayed in means after analysis of the last significant difference at 95% (LSD \leq 0.05) by Co-Stat Program (version 8.0).

RESULTS AND DISCUSSION

Results

Antifungal activity of plant extracts on the mycelial growth inhibition of the pathogenic fungi *in vitro*

Evaluation of plant extracts (aqueous by cold water) on root rot disease incidence was carried out under *in vitro* conditions. Data presented in Table (1) show that increasing all plant extracts

concentration decreased the mycelial linear growth of pathogenic fungi tested. Marjoram and basil were the most effective plant extracts against all soil-borne pathogenic fungi ranged (17.20 and 16.99% reduction mycelial linear growth respectively), while Peppermint and Spearmint were the least effective plant extracts against all soil-borne pathogenic fungi (12.84 and 4.61% respectively). According to rate plant extracts for their inhibitory effects using the scale, to determine the efficacy%, Basil, Marjoram and Peppermint were the moderately effective (++) against pathogenic fungi at 75 and 100% respectively, while Spearmint the slightly effect (+) at all concentrations.

Evaluation of plant extracts aqueous by (hot water) on pathogenic fungi were carried out under *in vitro* conditions. Data in Table (2) show that Marjoram plant extract was the most effective against soil-borne pathogenic fungi, ranged (13.18% reduction growth), followed by Basil, Peppermint (11.83 and 10.04% respectively), while Spearmint was the weak effective on pathogenic fungi (3.70%). Marjoram was the moderate effective against soil-borne pathogenic fungi (%Efficacy++) at high concentration (100%).

Effect of natural oils on mycelial growth inhibition *in vitro*

Different concentrations i. e., 25, 50, 75 and 100 ppm of three plant essential oils, *viz.* Garlic, Onion and Clove were evaluated for efficiency in suppressing growth of the soil-borne pathogenic fungi. Data in Table (3) show that all tested plant essential oils significantly reduced the mycelial linear growth of pathogenic fungi tested compared to control. The growth of the fungi decreased with increasing the concentration of plant essential oils. The most effective concentration was 500 ppm ranged (60.58% reduction growth), followed by 100 ppm (44.01%). While 50 ppm (30.78%). On the other hand 25 ppm was the least effective on growth of fungi (18.81%) compared with control. Garlic essential oil was the most effective against growth of pathogenic fungi ranged (36.02%), followed by clove oil (32.38%), while the least effect against growth was the onion oil (24.36%). According to rate of essential oils for their inhibitory effects using the scale, to determine the efficacy%, Garlic was effective against growth of pathogenic fungi at 100 and 500 ppm (+++ and +++), while clove moderately effective at 500 ppm (++) and the least effective was onion at 500 ppm(++) moderately effective.

Table (1): Effect of some plant extracts aqueous (cold water) on linear growth of pathogenic fungal *in vitro*.

Plant extracts	Conc. %	% Reduction mycelial linear growth				Mean	Efficacy
		<i>F. oxysporum</i>	<i>F. moniliforme</i>	<i>F. solani</i>	<i>B. theobromae</i>		
Basil	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	14.44	10.74	3.70	5.19	10.74	+
	50.00	27.78	16.67	9.26	7.78	16.30	+
	75.00	37.41	27.04	20.37	14.07	28.52	++
	100.00	46.30	32.59	25.19	18.89	40.00	++
Marjoram	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	9.63	9.26	8.89	8.89	7.04	+
	50.00	11.48	20.37	18.89	11.11	17.42	+
	75.00	27.79	28.89	29.63	27.78	29.26	+
	100.00	40.00	32.96	35.93	33.33	31.48	++
Peppermint	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	3.33	5.93	2.96	0.00	2.96	+
	50.00	6.67	17.41	13.33	8.52	14.07	+
	75.00	17.41	24.07	20.37	17.78	20.37	++
	100.00	28.15	23.33	28.15	19.63	29.26	++
Spearmint	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	0.00	0.00	0.00	0.00	0.00	-
	50.00	2.59	3.70	0.00	0.00	4.45	+
	75.00	7.78	7.78	2.96	5.93	5.19	+
	100.00	12.22	16.30	7.41	16.67	9.63	+
Mean		14.65	13.85	11.35	9.78	13.11	12.91

Table (2): Effect of some plant extracts aqueous (hot water) on linear growth of pathogenic fungal *in vitro*

Plant extracts	Conc. %	% Reduction mycelial linear growth				Mean	Efficacy %
		<i>F. oxysporum</i>	<i>F. moniliforme</i>	<i>F. solani</i>	<i>B. theobromae</i>		
Basil	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	10.37	4.44	2.22	2.22	8.15	6.30 +
	50.00	14.81	8.89	3.70	4.44	14.07	10.43 +
	75.00	25.19	13.70	14.81	12.22	22.59	20.00 18.09 +
	100.00	30.00	17.04	24.24	13.33	27.78	33.70 24.35 +
Marjoram	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	8.15	5.56	7.04	7.41	4.81	7.04 6.67 +
	50.00	11.11	13.70	12.96	9.26	14.44	16.30 12.96 +
	75.00	25.19	17.41	14.44	19.63	24.07	17.41 19.69 +
	100.00	36.67	20.74	21.85	30.33	25.19	24.81 26.60 ++
Peppermint	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	1.85	2.97	2.22	0.00	2.22	9.63 3.15 +
	50.00	3.07	13.70	11.48	6.30	12.59	15.19 10.39 +
	75.00	11.11	19.26	14.81	16.30	17.78	21.11 16.73 +
	100.00	18.15	20.37	20.00	18.15	19.63	23.33 19.94 +
Spearmint	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	0.00	0.00	0.00	0.00	0.00	-
	50.00	2.96	3.33	0.00	0.00	2.22	1.42 -
	75.00	8.89	4.44	2.22	4.81	3.70	13.33 6.23 +
	100.00	14.07	9.26	4.44	12.59	8.15	16.67 10.86 +
Mean		11.08	8.74	7.82	7.85	10.26	12.39 9.69

Table (3): Effect of some essential oils on linear growth of pathogenic fungal *in vitro*

Essential oils	Conc.ppm	% Reduction mycelial linear growth				Mean	Efficacy%
		<i>F. oxysporum</i>	<i>F. moniliforme</i>	<i>F. solani</i>	<i>B. theobromae</i>		
Garlic	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	25.56	22.22	2.97	19.63	21.85	26.67 19.82 +
	50.00	31.11	30.75	21.07	36.30	41.48	41.85 33.76 ++
	100.00	54.93	50.74	31.48	50.00	52.22	63.70 50.51 +++
	500.00	79.26	71.11	46.30	81.11	88.15	90.00 75.99 +++
	0.00	0.00	0.00	0.00	0.00	0.00	0.00 -
Onion	25.00	14.81	13.33	16.67	10.74	14.81	14.14 14.08 +
	50.00	24.81	28.89	27.28	24.44	19.63	27.78 25.47 ++
	100.00	35.56	29.26	38.45	36.67	36.67	37.04 35.56 ++
	500.00	44.07	37.04	51.11	47.41	49.26	51.11 46.67 ++
	0.00	0.00	0.00	0.00	0.00	0.00	0.00 -
	25.00	29.63	19.26	31.85	14.81	18.89	20.37 22.47 ++
Clove	50.00	42.41	27.41	39.26	28.52	27.78	31.85 32.87 ++
	100.00	56.67	48.89	53.33	40.00	38.89	47.04 47.47 ++
	500.00	83.70	54.07	61.11	49.63	50.74	55.19 59.07 +++
	Mean	34.83	28.86	28.04	29.28	30.69	33.78 30.92

Plant fixed oils, *viz.* Jojoba, Rocket and Sesame at different concentrations were evaluated for efficiency in suppressing mycelial growth of the soil-borne pathogenic fungi tested. Data in Table (4) show that Jojoba was the most effective against mycelial linear growth of pathogenic fungi ranged (35.99% reduction growth), followed by Rocket (21.06%), while the least effective on growth was Sesame (9.44%). On the other hand, all concentrations were significantly reduced the growth of pathogenic fungi, conc. 500 ppm ranged (45.76%) followed by conc. 100 ppm (31.26%), conc. 50 ppm (20.56%) and conc. 25 ppm (13.27%) compared with control.

Bioassay of commercial bioagents formulation on linear growth of pathogenic fungi *in vitro*

Four commercial bioagents formulations were tested to determine the most effective against soil-borne pathogenic fungi. Data in Table (5) indicate that Plant-Guard was the most effective against growth of pathogenic fungi ranged (41.93% reduction growth), followed by Rhizo-N (35.19%). On the other hand, Bio-arc and Bio-zeid were the least effective against growth of fungi (24.44 and 19.86%, respectively). However, mycelial linear growth was decreased with increasing the concentration of bioagents. Plant-Guard was the most effective at normal and high contractions (+++), followed by Rhizo-N (++) was moderately effective, while Bio-arc and Bio-zeid were the weak effective (++)�.

Chemical control

Effect of fungicides on mycelial linear growth of pathogenic fungi *in vitro*

Data presented in Table (6) indicate that all the tested fungicides reduced the mycelial linear growth of soil-borne pathogenic fungi *in vitro*. All the tested concentrations of fungicides significantly reduced the linear growth of tested fungi compared with the control treatment. Topsin M70 was the most effective fungicide against growth ranged (66.12% reduction growth), followed by kema-z (59.30%), the moderately effective were Tachigaren and Vitavax scored (53.02 and 46.75% respectively), while Moncut and Monceren fungicides were the least effective against growth of fungi (25.29 and 24.75%, respectively), compared with control.

Table (4): Effect of some fixed oils on linear growth of pathogenic fungal *in vitro*

Fixed oils	Conc.ppm	% Reduction mycelial linear growth						Mean	Efficacy%
		<i>F. oxysporum</i>	<i>F. moniliforme</i>	<i>F. solani</i>	<i>B. theobromae</i>	<i>T. Paradoxa</i>	<i>R. solani</i>		
Jojoba	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	21.48	19.26	15.56	30.37	19.26	24.81	21.79	+
	50.00	27.41	32.59	26.67	42.22	27.78	36.30	32.16	++
	100.00	52.96	61.85	38.15	56.67	35.93	58.89	50.74	+++
	500.00	84.81	86.30	56.67	90.37	52.96	80.37	75.25	+++
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
Rocket	25.00	2.97	14.81	15.93	18.15	16.67	20.37	14.82	+
	50.00	9.26	21.85	22.96	26.30	22.22	29.26	21.98	++
	100.00	19.63	30.74	24.07	36.30	27.78	36.67	29.20	++
	500.00	28.15	41.48	30.37	47.04	36.30	52.59	39.32	++
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	25.00	0.37	2.93	3.33	2.96	2.59	7.04	3.20	+
Sesame	50.00	4.81	7.41	5.93	6.30	7.41	13.33	7.53	+
	100.00	10.00	14.44	12.96	9.63	14.07	21.85	13.83	+
	500.00	14.81	22.96	19.26	14.14	22.22	42.59	22.66	++
	Mean	18.44	23.77	18.12	25.36	19.01	28.27	22.17	

Table (5): Effect of some bioagents commercial formulation on linear growth of pathogenic fungal *in vitro*.

Bioagents	Conc. / L	% Reduction mycelial linear growth					Mean	Efficacy %
		F. o.	F. m.	F. s.	B. t.	T. p.		
Bio-zeld (<i>Trichoderma album</i>)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	1.25 g	17.78	20.37	6.30	22.22	17.04	9.26	15.50 +
	2.5 g	28.15	30.74	17.04	38.89	29.26	18.15	27.04 ++
	3.50	41.11	38.89	26.67	50.00	39.63	25.19	36.92 ++
Bio-arc (<i>Bacillus megaterium</i>)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	1.25 g	19.63	30.74	16.67	27.78	20.37	18.89	22.35 ++
	2.5 g	30.74	41.85	32.96	35.19	28.89	28.52	33.03 ++
	3.5 g	38.52	50.74	42.22	44.07	39.63	39.26	42.41 ++
Rhizo-N (<i>Bacillus subtilis</i>)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	3 g	29.26	35.93	36.30	41.85	30.37	28.15	33.64 ++
	4 g	41.85	42.59	52.96	49.63	42.96	50.74	46.79 ++
	5 g	50.74	52.22	62.22	61.11	71.85	63.70	60.31 +++
Plant guard (<i>Trichoderma herzianum</i>)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	1.25 ml	59.63	41.85	45.93	39.26	38.15	39.26	44.01 ++
	2.5 ml	64.81	52.96	53.70	52.59	63.33	52.59	56.66 +++
	3.5 ml	70.37	63.70	64.44	58.15	73.33	72.22	67.04 +++
Mean		30.79	31.41	28.59	32.55	30.93	27.87	30.35

Table (6): Effect of some chemical fungicides on linear growth of pathogenic fungal *in vitro*.

Fungicide	Conc. ppm	% Reduction mycelial linear growth						Mean
		F. o.	F. m.	F. s.	B. t.	T. p.	R. s.	
Topsin M70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5.00	5.19	13.33	6.67	31.48	28.52	63.33	24.75
	10.00	10.37	27.78	21.11	61.48	49.63	100.00	45.06
	50.00	16.30	39.26	38.89	100.00	75.19	100.00	61.61
	100.00	25.19	58.15	51.85	100.00	100.00	100.00	72.53
	200.00	47.04	100.00	100.00	100.00	100.00	100.00	91.17
	400.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	500.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	1000.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Kema-Z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5.00	3.70	9.26	4.07	20.00	26.30	41.48	17.47
	10.00	9.26	17.04	15.19	57.78	46.67	73.70	36.61
	50.00	15.93	27.41	27.41	73.33	64.45	100.00	51.42
	100.00	24.81	42.22	39.26	100.00	74.81	100.00	63.52
	200.00	30.37	54.81	52.59	100.00	100.00	100.00	72.96
	400.00	50.37	100.00	100.00	100.00	100.00	100.00	91.73
	500.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	1000.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Vitavax	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5.00	3.33	8.15	5.56	11.67	20.37	38.89	14.66
	10.00	7.04	16.30	10.74	30.74	29.26	72.69	27.80
	50.00	12.59	24.45	16.67	51.85	41.11	100.00	41.11
	100.00	14.44	30.00	24.44	75.93	49.63	100.00	49.07
	200.00	21.11	50.37	36.67	100.00	62.22	100.00	61.73
	400.00	25.19	59.26	48.52	100.00	75.19	100.00	68.03
	500.00	36.30	69.26	58.52	100.00	100.00	100.00	77.35
	1000.00	42.96	75.96	66.67	100.00	100.00	100.00	80.93
Tachigaren	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5.00	5.93	9.26	7.41	8.89	30.74	35.19	16.24
	10.00	8.89	16.67	16.30	20.00	41.48	71.11	29.08
	50.00	15.55	27.40	27.04	39.26	52.96	100.00	43.70
	100.00	20.37	38.89	41.85	72.22	61.11	100.00	55.74
	200.00	27.41	51.11	51.11	100.00	72.59	100.00	67.04
	400.00	39.26	70.37	60.00	100.00	100.00	100.00	78.27
	500.00	50.37	100.00	72.59	100.00	100.00	100.00	87.16
	1000.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Moncut	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5.00	0.00	0.00	0.00	7.78	0.00	78.89	14.45
	10.00	0.00	0.00	0.00	16.30	0.00	100.00	19.38
	50.00	0.00	0.00	0.00	28.52	0.00	100.00	21.42
	100.00	0.00	0.00	0.00	41.48	0.00	100.00	23.58
	200.00	0.00	0.00	0.00	61.11	0.00	100.00	26.85
	400.00	0.00	0.00	0.00	75.19	15.93	100.00	31.85
	500.00	4.81	10.37	7.78	100.00	33.70	100.00	42.78
	1000.00	13.70	16.67	14.07	100.00	39.63	100.00	47.35
Monceren	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5.00	0.00	0.00	0.00	5.93	0.00	83.33	14.88
	10.00	0.00	0.00	0.00	14.07	0.00	100.00	19.01
	50.00	0.00	0.00	0.00	25.93	0.00	100.00	20.99
	100.00	0.00	0.00	0.00	38.89	10.00	100.00	24.82
	200.00	0.00	0.00	0.00	50.37	21.48	100.00	28.64
	400.00	0.00	0.00	0.00	58.15	32.96	100.00	31.85
	500.00	0.00	4.45	0.00	71.11	41.85	100.00	36.24
	1000.00	5.93	11.48	7.04	100.00	53.33	100.00	46.30
Mean		22.11	32.96	33.81	60.18	47.24	82.57	45.87

Date palm root rot diseases control with different treatment in greenhouse**1. Before inoculation with pathogenic fungi**

Results in Table (7) show that all tested fungicides and bioagents (biotic and abiotic) reduced disease severity of root rot disease on date palm seedlings var. Zaghloul. Soil drench with the tested materials three days before inoculation by the soil-borne pathogenic fungi proved to be good treatment in reducing disease severity compared with soil drench after three days of inoculation. Topsin M70 fungicide was the most effective against pathogenic fungi, *viz.* *F. oxysporum*, *F. monliforme*, *F. solani*, *T. paradoxa*, *B. theobromae* and *R. solani* under greenhouse conditions (87.68, 87.16, 86.62, 83.82, 72.65 and 59.30% reduction disease severity, respectively), followed by Kema-Z fungicide, while Jojoba fixed oil was moderately effective, followed by Vitavax, Tachigaren and Moncut, while Garlic essential oil, Plant-Guard bioagent biotic, Monceren fungicide and Marjoram plant extract were the least effective against disease severity, compared with control treatment. On the other hand, all the tested concentrations of different treatments were significantly reduced the disease severity of tested fungi compared to control treatment.

2. After inoculation with pathogenic fungi

Results in Table (8) show that all tested fungicides and bioagents (biotic and abiotic) reduced disease severity of root rot disease on date palm seedlings cv. Zaghloul. Soil drench with the tested materials three days after inoculation by the soil-borne pathogenic fungi proved to be good treatment in reducing disease severity compared with soil drench before three days of inoculation. Topsin M70 fungicide was the most effective to reduced disease severity against all fungi tested *viz.* *F. oxysporum*, *F. solani*, *F. monliforme*, *T. paradoxa*, *R. solani* and *B. theobromae* under greenhouse conditions (88.44, 85.75, 83.70, 80.94, 61.59 and 59.75% reduction disease severity, respectively), followed by Kema-Z, while Jojoba fixed oil, Tachigaren, Moncut and Vitavax fungicides were the moderately effective treatments against disease severity respectively. On the other hand, Garlic essential oil, Moncut fungicide, Marjoram plant extract and Plant-Guard biotic agent were the least effective against disease severity respectively, compared to control. On the other hand, high concentration was the most effective against disease severity.

Table (7): Effect of different methods of treatments on the disease severity of the root rot date palm caused by pathogenic fungi before inoculation.

Treatments	Conc.	% Disease Severity										R. s.	% Reduction
		F. o.	% Reduction	F. m	% Reduction	F. s.	% Reduction	B. t.	% Reduction	T. p.	% Reduction		
Topsin M70	0.00	31.08	0.00	22.75	0.00	11.58	57.77	8.75	34.80	6.25	74.14	8.17	29.99
	2.9 gm	11.50	63.00	10.83	52.40	11.50	57.77	6.92	48.44	5.83	75.88	6.25	46.44
Kema-Z	3.9 gm	7.25	76.67	6.58	71.08	6.92	74.76	6.92	72.65	3.91	83.82	4.75	59.30
	4.9 gm	3.08	87.68	2.92	87.16	3.67	86.62	0.00	13.42	0.00	24.17	0.00	11.67
Vitavax	0.00	31.08	0.00	22.75	0.00	11.75	50.55	9.17	31.67	8.25	65.87	8.58	26.48
	2.9 gm	12.08	61.13	11.25	50.55	11.75	57.15	7.42	72.94	40.98	6.42	44.99	44.99
Tachigaren	4.9 gm	9.17	70.50	6.83	69.98	6.92	77.93	5.92	55.89	4.75	80.35	4.92	57.94
	0.00	31.08	0.00	22.75	0.00	27.42	0.00	13.42	0.00	24.17	0.00	11.67	0.00
Moncut	2.9 gm	13.08	57.92	12.92	43.21	13.50	50.77	11.42	14.90	10.08	58.30	10.58	9.34
	4.9 gm	10.83	65.15	10.58	53.49	11.33	58.68	9.42	29.81	8.67	64.13	8.67	25.71
Garlic	8.42	72.91	8.33	63.38	9.33	65.97	7.58	43.52	6.75	72.07	6.92	40.70	40.70
	0.00	31.08	0.00	22.75	0.00	27.42	0.00	13.42	0.00	24.17	0.00	11.67	0.00
Marjoram	2.9 gm	13.75	55.76	13.42	41.01	14.33	47.74	10.83	19.30	9.42	61.03	9.17	21.42
	4.9 gm	11.25	63.80	11.33	50.20	11.92	56.53	9.17	31.67	7.83	67.60	8.25	29.31
Jojoba	0.00	31.08	0.00	22.75	0.00	27.42	0.00	13.42	0.00	24.17	0.00	11.67	0.00
	50 ppm	15.42	50.39	15.75	30.77	16.25	40.74	9.25	31.07	10.25	57.59	7.67	34.28
Plant guard	100 ppm	11.58	62.74	13.25	41.76	14.08	48.65	8.08	39.79	7.33	69.67	5.83	50.04
	250 ppm	8.17	73.71	12.33	45.80	12.92	52.88	6.92	48.44	6.50	73.11	4.17	64.27
L. S. D. at 0.05%	Fungi (F)	0.23	F X T	0.72	F X C	0.45	T X C	0.18	F X T X C	1.43			
	Treatments (T)	0.29											
	Concentrations (C)	0.18											
Mean		17.21		14.96		16.62		9.46		11.70		8.22	

Table (8): Effect of different methods of treatments on the disease severity of the root rot date palm caused by pathogenic fungi after inoculation.

Treatments	Conc.	% Disease Severity											
		F. o.	% Reduction	F. m	% Reduction	F. s.	% Reduction	B. T.	% Reduction	T. p.	% Reduction	R. s.	% Reduction
Topsin M70	0.00	38.25	0.00	25.58	0.00	33.33	0.00	15.33	0.00	30.58	0.00	13.67	0.00
	2 gm	13.42	64.92	12.25	52.11	14.58	56.26	10.92	28.77	9.17	70.01	9.83	28.09
Kema-Z	4 gm	8.08	78.88	8.50	66.77	8.17	75.49	9.25	39.66	8.00	73.84	6.75	50.62
	0.00	38.25	4.42	88.44	4.47	83.70	4.75	85.75	6.17	59.75	5.83	80.94	5.25
Vitavax	2 gm	12.67	66.88	12.08	52.78	13.17	60.49	11.17	27.14	9.17	70.01	9.17	32.92
	3 gm	10.17	73.41	7.75	69.70	9.33	72.01	9.83	35.88	7.75	74.66	6.67	51.21
Tachigaren	4 gm	7.08	81.49	6.50	74.59	7.42	77.74	5.92	54.86	5.92	80.64	5.42	60.35
	0.00	38.25	0.00	25.58	0.00	33.33	0.00	15.33	0.00	30.58	0.00	13.67	0.00
Monconer	2 gm	14.08	63.19	13.75	46.25	16.17	51.49	14.50	5.41	11.58	62.13	12.75	6.73
	3 gm	11.58	69.73	11.58	54.73	14.17	57.49	13.58	11.42	10.17	66.74	9.50	30.50
Garlic	2 gm	9.17	76.03	10.08	60.59	10.42	68.74	10.33	32.62	7.08	76.85	7.58	44.55
	0.00	38.25	0.00	25.58	0.00	33.33	0.00	15.33	0.00	30.58	0.00	13.67	0.00
Jojoba	50 ppm	75.00	46.85	19.17	25.06	20.83	37.50	11.17	27.14	10.08	67.04	10.83	20.78
	100 ppm	16.83	56.00	16.92	33.85	34.21	18.75	43.74	14.25	7.05	12.58	58.96	8.17
Plant guard	50 ppm	38.25	0.00	25.58	0.00	33.33	0.00	15.33	0.00	30.58	0.00	13.67	0.00
	100 ppm	17.08	55.35	18.67	27.01	21.00	36.99	11.33	26.09	10.42	65.93	9.25	32.33
Mean	1.25 gm	37.50	1.96	20.83	18.57	30.83	7.50	43.23	9.33	39.14	8.83	42.72	7.83
	2.5 gm	29.92	21.78	19.25	24.75	25.58	23.25	11.33	14.17	7.57	16.25	46.86	12.83
	3.5 gm	25.58	33.12	18.08	29.32	24.17	27.48	9.25	39.66	10.92	54.22	8.92	34.75
	L. S. D. at 0.05%	21.08	16.78	20.36	11.66	14.76	11.66	14.76	11.66	14.76	9.63	5.50	52.45

Fungi (F)
Treatments (T)
Concentrations (C)

FXT
FXC
TXC

Discussion

Date palm trees and offshoots are subjected to infection with different diseases caused by many fungi causing considerable root rot in the orchard trees (El-Deep, 1994, Barka *et al.*, 2011). The aim of this study was to examine some plant extracts, natural oils, biofungicides and fungicides against root rot disease of date palm. The efficacy of different plant leaf extracts (basil, marjoram, peppermint and spearmint) against soil-borne fungi of root rot date palm were tested *in vitro* and *in vivo*. The results showed that the three (basil, marjoram, peppermint) plant leaf extracts (prepared by cold water) were significantly ($P \leq 0.05$) inhibited the radial growth of all the test fungi with inhibition varying from one extract to another. Percentage inhibition of radial growth of all the fungi was highest in marjoram extract and lowest in extract from spearmint. Akinpelu (1999) reported the water-soluble antifungal principles in the plants as being responsible for the anti-fungal activities; also, Olufolaji (1999) used aqueous plant extracts in the control of wet rot of *Amaranthus* sp. caused by *Choanephora cocurbitarum*. Suleiman *et al.* (2008) mentioned that vegetative growth values for *Fusarium* sp. at different concentrations of *Senna alata* extract were generally low compared to control. All the leaf extracts were effective in the reduction of the incidence of all the soil-borne pathogenic fungi tested except extracts from spearmint that did not give significant ($P \leq 0.05$) reduction of the fungi when compared with untreated control seedlings. This result indicates that the leaf extracts of basil and marjoram probably have some fungicidal properties that inhibit the growth of the soil-borne fungi. The crude extracts were more effective in reducing the incidence of fungi than the aqueous extracts. This is an indication that dilution of the extracts reduced toxic effects of the leaf extracts on the soil-borne fungi. These results agrees with the findings of Zaman *et al.* (1997), they reported that the efficacy of garlic, neem, ginger and onion extracts on seed borne fungi of mustard declined with increase dilution. The cold water extraction method was the most effective way in promoting the action of plant extraction compared to hot water

extraction method. Bansal and Gupta (2000) and Srivastava *et al.* (2010) studied and reported that aqueous leaf extracts of some medical plants were tested against *Fusarium oxysporum*. These findings contradicted with those of Shukla, *et al.* (2002) they reported that ether extract was more effective in reducing the population of *Alternaria alternata* and *Fusarium palliodoroseum* than the boiling water extract. Essential oils were tested against pathogenic fungi *in vitro* and greenhouse. The general categories of plant natural products are as follows (Kaufman *et al.*, 1999): 1) the lipids, including the simple and functionalized hydrocarbons, as well as terpenes; 2) aromatic compounds, including phenols; 3) carbohydrates; 4) amines, amino acids, and proteins; 5) alkaloids; and 6) nucleosides, nucleotides, and nucleic acids. Garlic was found to be the most effective against mycelial growth of all the pathogenic fungi tested. These results were in harmony with those obtained by (Jularat *et al.*, 2009 and Tanović, *et al.*, 2009). The mode of action in garlic oil by the active compounds of garlic (sulfur compounds) which destroyed the fungal cells, includes decreasing the oxygen uptake, reducing cellular growth, inhibiting the synthesis of lipids, proteins and nucleic acids, changing the lipid profile of the cell membrane and inhibiting the synthesis of the fungal cell wall (Gupta and Porter, 2001). Fiori *et al.* (2000) and Farid *et al.* (2002) reported that the efficacy of medicinal plants against various species of pathogenic fungi were dependent on the origin of the medicinal plant and the sensitivity of the target fungi. The present results showed that essential oils of the plants; onion (*Allium cepa* L.), garlic (*Allium sativum* L.) and clove (*Syzygium aromaticum* L.) are able to inhibit the mycelium growth of *F. oxysporum*, *F. solani*, *F. moniliforme*, *T. paradoxa*, *B. theobromae* and *R. solani*, which is a clear indication of begin a potential alternative source of synthetic fungicides to control these pathogenic fungi. Fixed oils were tested against mycelial growth. Jojoba oil was the most effective under *in vitro* and *in vivo* conditions against pathogenic fungi tested. Jojoba oil is a straight-chain wax ester of 36 to 46 carbons in length, an ester bond in the approximate middle of the chain, used as antimicrobial. Such results are in agreement with those

reported by Leonard and Stephen (2007). Many biological control agents perform poorly under field conditions and only few biocontrol species have been registered for field use. Biocontrol of soil-borne pathogens has been more successful under controlled environmental conditions using simplified potting mixtures presumably low in microbial diversity (Fravel, 1999; Copping, 2001). Biological control agents may use a variety of inhibitory and suppressive mechanisms: (1) competition for resources and space, (2) antibiotic production, (3) removal of pathogenicity factors produced by the pathogen, (4) production of degrading enzymes that target the pathogen and (5) the induction of resistance in the host plant (Whipps, 2001). Biological activity of antagonist fungi and bacteria may partially be associated with production of antibiotic (Etebarian *et al.*, 2000). The production of antibiotics were; Trichodermin (Godtfredsen and Vangedal, 1964), ergokonin (Kumeda *et al.*, 1994). Biological control of fungal plant pathogens appears as an attractive and realistic approach, and numerous microorganisms have been identified as biocontrol agents. *Trichoderma harzianum* and *Bacillus subtilis*, which are common saprophytic fungi found in almost any soil and rhizosphere micro flora in all governorates under study, have been investigated as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly many common soil borne pathogens, these findings were in agreement with (El-Katatny *et al.*, 2006; Dubey *et al.*, 2007). All fungicides tested in the laboratory significantly reduced pathogen development when compared with the control. Treatment with Topsin M70 had the greatest inhibitory effect on mycelial growth and re-growth at all concentrations (5, 10, 50, 100, 500 and 1000 ppm). Inhibitory effect of Topsin M70 against many plant soil-borne pathogenic fungi has been reported by many researchers. (Ammar, 2003 and Korra, 2005). Under greenhouse conditions, Marjoram, Garlic and Jojoba were the most effective as abiotic agents to reduce the disease incidence and disease severity. These results are agree with (Curtis *et al.*, 2004; Satya *et al.*, 2005). On the other hand, biotic agent's, *viz.* *Trichoderma harzianum* and *B. subtilis* were the most effective to reduce

disease incidence and severity. These results agree with those reported by (Boyd-wilson *et al.*, 2000). Also, (Harman, 2006; Abd-El-Khair, *et al.*, 2010) they reported that *Trichoderma* spp. utilize various mechanisms including nutrient competition, antibiosis, antagonism, inhibition of pathogen or plant enzymes; processes of biodegradation, carbon and nitrogen cycling; complex interactions with plants in the root zone of the rhizosphere, which involve various processes such as colonization, plant growth stimulation, bio-control of diverse plant pathogens, decomposition of organic matter, symbiosis and nutrient exchange. Topsin M70 fungicide was the most effective to reduce the root rot disease of date palm under greenhouse conditions. These results agree with those reported by (Rashed, 1998; Srivastava, *et al.*, 2010) they reported that Topsin M70, Kema-Z and Kocide 101 were the best for the therapy of palm trees diseases especially black scorch disease. The difference gained among the different pesticides under study could be attributed to one or more factors, as mode of action of the fungal cell (Watkins, *et al.*, 1977), degree of permeability of cell wall and/or plasma-lemma of fungi for uptake and passage of the fungicide into the fungal cell (Giffin, 1981), chemical composition of the fungicide (Carnegia, *et al.*, 1990). Application time of the fungicides played a role in reducing diseases severity of date palm root rot. The results of the present study revealed that spraying the fungicides before pathogen inoculation caused more reduction in the disease severity. Such results are in agreement with those reported by El-Morsi (1999); Ammar (2003); Molan *et al.* (2003). Finally, this study demonstrate the efficacy of certain abiotic and biotic agents and fungicides for controlling several pathogenic fungi that cause the root rot diseases of date palm, which may be applied in an integrated control program for management of date palm pests and diseases in Egypt.

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كفاءة بعض المستخلصات النباتية والزيوت الطبيعية والمركبات الحيوية والمبيدات الفطرية ضد مرض عفن جذور نخيل البلح

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تم عزل العديد من فطريات التربة من اعفان جذور اشجار وسائل نخيل البلح وشملت الفطريات الفطر فيوزاريوم اوكتسيبورم، والفطر فيوزاريوم مونيليفورم، والفطر فيوزاريوم سولاني، والفطر ثيلافيسوس بارادوكسا، والفطر ريزوكتونيا سولاني. فى التجارب المعملية لتقدير فعالية بعض المستخلصات النباتية كان مستخلص البردقوش بتركيز 100% اكبر المستخلصات فعالية ضد الفطريات المختبرة، بينما الزيت الطيار للثوم عند تركيز 500 جزء فى المليون والزيت الثابت للجوجوبا عند تركيز 500 جزء فى المليون اكثراً الزيوت فعالية. المركب الحيوى بلانت جارد عند تركيز 3.5 مل/لتر كان اكثراً المركبات فعالية ضد الفطريات المرضية فى المعمل، بينما المبيد الفطرى توبisin ام 70 كان اكثراً المبيدات الفطرية فى المعمل. فى تجارب الصوبة وجد ان المبيد الفطرى توبisin ام 70 كان اكثراً المعاملات فاعلية فى تقليل مرض عفن جذور النخيل.